



nium sp. appearing to be a pioneer stage and *Corallina* sp., the climax one. Further study will be needed to fill in the serial stages. This could be significantly related to the gradation in phototaxis and niche separation between size classes.

The gradation in activity and phototactic response in *Mopalia muscosa* between the relatively unworn valves of the young, to the badly worn (or even algae covered) valves of the older chitons is similar to results of CROZIER (1921) in *Chiton tuberculatus*. GLYNN (1970) found a difference on the interspecific level between *C. tuberculatus* and *Acanthopleura granulata* (Gmelin, 1791) as to the time spent feeding. This behavior would give an effect of dividing the age classes into different niches and reducing intraspecific competition in *M. muscosa* with greater utilization of the habitat.

This is further shown by the night field observations during which numerous small chitons were seen out on the floors and sides of shallow tide pools. These were very rarely seen during day observations. This would follow the behavior of negative phototactic responses of the smaller chitons found during the experiment.

Differences in vertical distribution, due to size classes, have been described by FRANK (1965) for *Acmaea digitalis* Rathke, 1833. Similar findings for *M. muscosa* were observed, with the largest chitons occupying the highest vertical zone.

An association was observed with the gastropod *Tegula funebris* (A. Adams, 1855) always clustered around the group of chitons previously described as the largest and completely covered by algae (Figure 2). This relationship is most likely only a means of conserving moisture in the microclimate created by clustering, thus giving mutual benefit with *T. funebris* taking the active role in clustering. The substrate was almost void of vegetation and with a limited amount of shallow tide pools and ledges. Therefore this association is an utilization of the cover, afforded by the chitons, from desiccation. This would aid the large

chitons to endure aerial exposure, since they move the least of the classes and are often exposed up to eight hours.

ACKNOWLEDGMENTS

The study was originally done while I was at San Francisco State University. I am grateful to Dr. Gary Vermeij, Dr. James A. Marsh, Dr. Lucius Eldredge, and Frieda Osborne for their constructive criticisms of the original manuscript. I'm also very grateful to Dr. Roy T. Tsuda for his advice and criticism in the preparation of the paper.

Literature Cited

- AREY, L. & W. CROZIER
1919. Sensory responses of *Chiton*. Journ. Exp. Zool. 29: 583 - 596
- BECKETT, T. W.
1968. Limpet Movements. An investigation into some aspects of limpet movements, especially the homing behaviors. Tane 14: 43 - 64
- CROZIER, W. J.
1920. Note on the photic sensitivity of the chitons. Amer. Nat. 54: 376 - 380 (July-August 1920)
1921. Homing behavior in chiton. Amer. Nat. 55: 276 - 281; 1 text fig. (May-June 1921)
- FRANK, PETER WOLFGANG
1965. The biodemography of an intertidal snail population. Ecology 46 (6): 831 - 844; 8 figs.; 6 tables
- GALBRAITH, ROBERT T.
1965. Homing behavior in the limpets *Acmaea digitalis* and *Lottia gigantea*. Amer. Midl. Natural. 74 (1): 245 - 246
- GLYNN, P. W.
1970. On the ecology of the Caribbean chitons *Acanthopleura granulata* and *Chiton tuberculatus*. Smithson. Contr. Zool. 66
- HYMAN, LIBBIE HENRIETTA
1967. The invertebrates, vol. 6: Mollusca I. McGraw-Hill, New York, N Y, i - vii, 1 - 792; 249 figs.
- LEWIS, JOHN ROBERT
1964. The ecology of rocky shores. v + 323 pp.; 40 pls.; 85 text figs. London, English Univ. Press Ltd.
- TEST, AVERY RANSOME
1945. Ecology of California *Acmaea*. Ecology 26 (4): 395 - 405
- THORNE, M. J.
1968. Studies on homing in chiton *Acanthozostera gemmata*. Austral. Mar. Freshwater Res. 19: 151 - 160
- TUCKER, JOHN S. & ARTHUR CHARLES GIESE
1959. Shell repair in chitons. Biol. Bull. 166: 318 - 322
- WELLS, MARY JANE
1965. Learning by marine invertebrates. Adv. Mar. Biol. 3: 1 - 62 Acad. Press Inc., New York



An Anomalous Style in the Gut of *Megatebennus bimaculatus*, a Carnivorous Prosobranch Gastropod

BY

MICHAEL T. GHISELIN, ELAINE DE MAN

University of California Bodega Marine Laboratory, P. O. Box 247, Bodega Bay, California 94923

AND JOHN P. WOURMS

New York Ocean Science Laboratory, P. O. Drawer EE, Montauk, New York 11954

(1 Plate)

THE CRYSTALLINE STYLE is a rod of mucoprotein and other materials occurring in the intestine and stomach of certain mollusks, mainly bivalves (BEDFORD & RIED, 1969; MICHELSON & DUBOIS, 1971). Its function remains controversial, but it is thought to aid in the movement and trituration of food, and in the release of enzymes. YONGE (1930, 1932) drew attention to the correlation between the kind of food eaten and the presence of a style: it appeared to be restricted to forms which eat food of plant origin. He proposed a simple and plausible explanation for this correlation. A carnivore would need an extracellular protease, but this would digest the style itself. Hence their coexistence would be impossible. This hypothesis is questionable on the grounds that many carnivores have adaptations which prevent their extracellular proteases from digesting their own guts. One must admit, however, that for some unknown reason mollusks were never able to evolve such an adaptation for the style. Polemics over whether style-bearing forms have extracellular proteases have thus far proved indecisive (YONGE, 1946; MANSOUR, 1946; MANSOUR-BEK, 1946). A style-bearing neogastropod, *Nassarius obsoletus* (Say, 1822) has carnivorous relatives, but is found to eat both plants and animals (JENNER, 1956; see also MORTON, 1960). BROWN (1969) found no protease in the style of this species, and correctly pointed out that this can be explained in terms of Yonge's hypothesis. However, such a correlation is never decisive.

A related correlation has to do with the distribution of "oesophageal glands" in gastropods (YONGE, 1932; GRAHAM, 1939; FRETTER & GRAHAM, 1962). These structures secrete proteases. They are supposed to be present in many carnivores and absent in herbivores. FRETTER &

GRAHAM (1962:637) assert that "The alimentary canal of opisthobranchs is invariably characterized by the complete absence of any structures which can be homologized with the oesophageal glands of prosobranchs." GHISELIN (1963) suggested a possible homologue among herbivorous cephalaspideans, but this has no bearing upon the physiological issues. Gastropods with oesophageal glands are said never to have a style, again because it would be digested.

Megatebennus bimaculatus (Dall, 1871) is a gastropod of the family Fissurellidae, the "keyhole limpets." Other members of its family have been said to be either herbivores or carnivores. Some, but by no means all, have an entity somewhat resembling a crystalline style, but up to the present it has been dismissed as something other than a true crystalline style. *Megatebennus bimaculatus* definitely is a carnivore, for we have seen it feeding on compound tunicates in the laboratory. It tends to be associated with tunicates in the field. The shell has become vestigial, and a polymorphic cryptic color pattern is evident; the snails closely resemble various compound ascidians. To be sure, it cannot be demonstrated that they never eat plant materials. We have occasionally found sponge spicules and sand in the gut.

A well-developed style (Figure 1) resembling the crystalline style of many bivalves (see especially KRISTENSEN, 1972) and large oesophageal glands are present in this species. A simple but definitive spot test for proteases (see WELSH *et al.*, 1968) showed that they are present in the digestive gland, gastric fluid, oesophageal gland, and in the style itself, but not in non-digestive tissues and haemocoelic fluid used as controls. The style-sac, thought to secrete the style, is open to the intestine; as this con-

nection might allow incorporation of the enzyme from the rest of the gut, the origin of the style protease remains uncertain. Yet it should be all the more evident that the style is readily accessible to the action of the enzymes.

The foregoing observations refute the hypothesis that a carnivorous diet and extracellular proteases make the presence of a crystalline style impossible. Nonetheless the empirical rule holds true: a crystalline style and a carnivorous habit can, but normally do not, coexist. The same applies to the oesophageal glands.

One might contend that there is something unusual about the style of *Megatebennus bimaculatus*, but it seems more reasonable to infer that the reason for the correlation is that a carnivore derives little advantage from possessing a style. If so, it is likely that *M. bimaculatus* is in a sense a phylogenetic relic—a member of an herbivorous or omnivorous stock in the process of becoming carnivorous, and hence possessing a mixture of ancestral and derived features.

Alternatively one might say that the entity we have been dealing with really is not a crystalline style at all, and this has in fact been the prevailing view with respect to comparable structures in other fissurellids. They are considered to have, if anything, a "protostyle," representing an early stage in the evolutionary development of a crystalline style (GRAHAM, 1939; WARD, 1966). Discussions about what to call such entities are complicated by semantic issues, circular reasoning, and the confusion of fact with hypothesis. GRAHAM (1939:77) defined "crystalline style" as one lacking amylases. There is no particular reason for defining the term in this fashion, and it is rather unfortunate as it would prejudice the case against forms which secrete amylase elsewhere or not at all. In evolutionary anatomy there is every reason to designate homologues with the same name. He also found a rod of mucous material in the intestine of *Diodora apertura*, a carnivorous fissurellid. His tests for amylase were negative, and he dismissed this structure as merely the first beginnings of a fecal rod.

Owen's perhaps too cursory observations may have influenced later thought on the so-called protostyle. Vestigial structures and laboratory artifacts could easily have been misinterpreted. In addition, efforts to arrange the gastropods in an old-fashioned scale from "lower" to "higher" may have biased the interpretations. Be this as it may, MORTON (1952:86) defined "protostyle" as "a rod of compressed faeces, contained within the style sac, rotated backwards into the intestine by obliquely transverse ciliary currents, and not containing an amylolytic enzyme." He provided some information on an herbivorous fissurellid, *Scutus breviculus*, to exemplify an archaeogastropod

with a protostyle. OWEN (1958) found extracellular amylase, protease and lipase in this organism, got negative results with efforts to find uptake of iron saccharate in the digestive gland, and concluded that digestion is extracellular. He inferred that the digestive apparatus has undergone secondary simplification.

To see if any amylase is present in *Megatebennus bimaculatus*, routine spot tests (WELSH *et al.*, 1968) were carried out on seawater extracts of style, style sac, stomach, salivary glands, oesophageal glands and digestive glands, and on fluid removed from the lumen of the stomach. All except the style itself were positive; the negative result of course is not demonstrative. Our findings with respect to the oesophageal glands differ from those of WARD (1966) on *Fissurella barbadensis* Gmelin, 1791 who found amylase, but no protease.

To see whether the *Megatebennus bimaculatus* style fits the descriptions of protostyles or crystalline styles from other mollusks, specimens were examined by light and electron microscopy. For further information on molluscan style ultrastructure and biochemistry see papers by WOURMS (1968, 1970), WOURMS *et al.* (in manuscript) and WONG *et al.* (in manuscript).

The style of *Megatebennus bimaculatus* (Figure 1) is a hyaline rod of mucus-like material, with a soft, dark core. The core contains material resembling the partly digested food observed in the stomach. The outer, clear portion is fairly thick, and definitely does not contain faeces. Thus the style, like that of many mollusks in which the style sac communicates with the intestine, evidently takes up material at the end away from the stomach. As the style moves into the stomach, where it breaks down, the style sac epithelium secretes the more hyaline portion around the central core. Therefore neither as to structure or to function does this style fit MORTON'S (1953:241) characterization of the protostyle as a "rod of stiff viscid mucus, studded with faeces derived both from waste material of the digestive gland, and from larger indigestible particles rejected by the stomach." All the evidence indicates that the style materials are secreted, moved and dissolved as in crystalline styles.

Styles of *Megatebennus bimaculatus* were prepared for ultrastructural analysis by standard techniques (see DAWES, 1971; SJÖSTRAND, 1967). Segments of styles were fixed at 4° C for 6 hours in 2.5% glutaraldehyde, 3.5% sucrose in 0.1 M Sorenson's phosphate buffer at pH 7.3. After initial fixation, the segments were washed in buffered sucrose and post-fixed for 11 hours in cold 2% osmium tetroxide in 3.5% sucrose, 0.1M phosphate buffer (pH 7.3). Following rapid dehydration through a graded series of acetone, the style segments were embedded in Luft's